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Terms: **author (solomon) and title (endothelial)** ([Edit Search](#))

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**TITL:** The seeding of human aortic **endothelial** cells on the extra-cellular matrix of human umbilical vein **endothelial** cells.

**AUTH:** Solomon D E

**ORGA:** Research Division, Miami Heart Institute, FL 33140.

PUB TYPE: Journal Article.

**CITE:** Int J Exp Pathol 1992 Aug; 73 (4): 491-501

**LANG:** ENG; English

**ABST:** A post confluent layer (6th passage) of human umbilical vein endothelial cells (HUVECs) was treated with 3 mM ethylene diamine tetra-acetic acid (EDTA) to expose the subendothelial extra-cellular matrix (ECM). Normal human aortic endothelial cells (HAECs) harvested by mechanical scraping were seeded onto the ECM of the HUVECs. The cells quickly attached and proliferated with normal morphology. To ensure confluency the HAECs were pooled after a brief trypsin/EDTA incubation and seeded onto the ECM of the same HUVECs (6th passage) cell line. They attached within 2 hours, and the cells grew to confluence displaying cobblestone morphology characteristic of phenotypic endothelium. HUVECs (11th passage) were seeded onto (6th passage) HUVECs ECM. The cells attached, proliferated to confluence within the normal time interval (7-8 days) and were positively characterized. A Corvita 6mm graft supplied with a gelatin/heparin matrix was densely seeded with HUVECs (6th passage). These cells also proliferated to confluence. The implications for improving the design of arterial grafts are discussed.

**MJTR:** Aorta, cytology. Arteries, transplantation. Endothelium, Vascular, cytology. Extracellular Matrix. Umbilical Veins, ultrastructure.


**MNTR:** Cell Adhesion. Cell Division. Cells, Cultured. Culture Media. Human. Tissue Culture.

**RNUM:** 0 (Culture Media)

**GEOT:** ENGLAND

**IDEN:** ISSN: 0959-9673. JOURNAL-CODE: 9014042. ENTRY-DATE: 19921102. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.


**PMID:** 1390196

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Terms: **author (solomon) and title (endothelial)** ([Edit Search](#))

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Terms: author (cai and ye) and title (mcp) ([Edit Search](#))

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**TITLE:** The intracellular signaling pathways involved in **MCP-1**-stimulated T cell migration across microvascular endothelium.

**AUTH:** Cai J P; Hudson S; Ye M W; Chin Y H

**ORGA:** Department of Microbiology, University of Miami School of Medicine, Florida 33101, USA.

**PUB TYPE:** Journal Article.

**CITE:** Cell Immunol 1996 Feb 1; 167 (2): 269-75

**LANG:** ENG; English

**ABST:** The adherence and transmigration of T cells through microvascular endothelium is an essential step for recruitment into inflammatory lesions, although the factors that stimulate the directional migration of T cells have not been fully characterized. In the present study we investigated the capacity of chemokines to induce migration of T cells across dermal microvascular endothelial cell monolayer. The results showed that recombinant MCP-1 significantly induced transendothelial migration of both resting and activated T cells. Maximal induction of migration was observed at a concentration of 10 ng/ml and a 3- to 4-hr incubation period. In contrast, the chemokines IL-8, RANTES, and MIP-1 alpha failed to stimulate T cell migration at doses as high as 100 ng/ml. In studies designed to investigate the intracellular signaling pathways mediating the MCP-1 effect, the results showed that MCP-1 at doses ranging from 10 to 100 ng/ml did not cause an increase in intracellular calcium ions in T cells, even though this chemokine induced rapid calcium mobilization in monocytes. Furthermore, pretreatment of T cells with either bisindolymaleimide HCl, a specific inhibitor of protein kinase C, or genistein, a protein tyrosine kinase inhibitor, significantly decreased the MCP-1-induced transmigration in a dose-dependent manner. In contrast, T cells pretreated with the protein kinase A-specific inhibitor H89 responded normally to MCP-1 stimulation. Finally, T cell transmigration was inhibited by antibodies against CD11a, thereby confirming the importance of beta 2-integrin in the transmigration process.

**MJTR:** Endothelium, Vascular, cytology. Monocyte Chemoattractant Protein-1, physiology. T-Lymphocytes, cytology.

**MNTR:** Calcium, metabolism. Cell Movement. Cells, Cultured. Chemotaxis, Leukocyte. Cyclic AMP-Dependent Protein Kinases, antagonists & inhibitors. Cyclic AMP-Dependent Protein Kinases, physiology. Enzyme Inhibitors, pharmacology. Human. Interleukin-8, physiology. Lymphocyte Activation. Lymphocyte Function-Associated Antigen-1, physiology. Macrophage Inflammatory Protein-1. Monocytes, physiology. Monokines, physiology. Protein Kinase C, antagonists & inhibitors. Protein Kinase C, physiology. Protein-Tyrosine Kinase, antagonists & inhibitors. Protein-Tyrosine Kinase, physiology. RANTES, physiology. Skin, blood supply. Support, Non-U.S. Gov't. Support, U.S. Gov't, P.H.S..


**RNUM:** 0 (Enzyme Inhibitors); 0 (Interleukin-8); 0 (Lymphocyte Function-Associated Antigen-1); 0 (Macrophage Inflammatory Protein-1); 0 (Monocyte Chemoattractant Protein-1); 0 (Monokines); 0 (RANTES); 7440-70-2 (Calcium); EC 2.7.1.112 (Protein-Tyrosine Kinase); EC 2.7.1.37 (Cyclic AMP-Dependent Protein Kinases); EC 2.7.1.37 (Protein Kinase

C)

**GEOT:** UNITED STATES

**IDEN:** ISSN: 0008-8749. JOURNAL-CODE: 1246405. ENTRY-DATE: 19960515. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

**PMID:** 8603436

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Terms: **author (cai and ye) and title (mcp)** ([Edit Search](#))

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Terms: **author (kramer and bensch)** ([Edit Search](#))

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**TITLE:** Synthesis of extracellular matrix glycoproteins by cultured microvascular endothelial cells isolated from the dermis of neonatal and adult skin.

**AUTH:** **Kramer** R H; **Fuh** G M; **Bensch** K G; **Karasek** M A

PUB TYPE: Journal Article.

**CITE:** J Cell Physiol 1985 Apr; 123 (1): 1-9

**LANG:** ENG; English

**ABST:** We examined the synthesis of extracellular matrix macromolecules by human microvascular endothelial cells isolated from the dermis of neonatal (foreskin) and adult (abdominal) skin. Electron microscopy showed that both cell types produced an extracellular matrix that was strictly localized to the subendothelial space. The subendothelial matrices were initially deposited as a single discontinuous layer of filamentous, electron-dense material that progressively became multilayered. Biosynthetic studies indicated that 2-4% of the newly synthesized protein was deposited in the subendothelial matrices by both cell types. Approximately 15-20% of the radiolabeled protein was secreted into the culture medium, and the remainder was confined to the cellular compartment. Biochemical and immunochemical analyses demonstrated the extracellular secretion of type IV collagen, laminin, fibronectin, and thrombospondin by the newborn and adult cells. Whereas type IV collagen was the predominant constituent of the matrix, fibronectin was secreted into the medium, with only small amounts being deposited in the matrix. Thrombospondin was a major constituent of the matrix produced by the newborn foreskin cells but was virtually absent in the matrix elaborated by the adult cells. However, both cell types did release comparable amounts of thrombospondin into their medium. Immunoperoxidase staining for type IV collagen revealed a fibrillar network in the subendothelial matrices produced by both adult and neonatal cells. In contrast, thrombospondin, which was detected only in the matrix of newborn cells, exhibited a spotty and granular staining pattern. The results indicate that the extracellular matrices synthesized by cultured human microvascular endothelial cells isolated from anatomically distinct sites and different stages of development and age are similar in ultrastructure but differ in their macromolecular composition.

**MJTR:** Endothelium, metabolism. Extracellular Matrix, metabolism. Glycoproteins, biosynthesis. Skin, blood supply.

**MNTR:** Aging. Cells, Cultured. Collagen, analysis. Collagen, biosynthesis. Comparative Study. Endothelium, analysis. Endothelium, cytology. Extracellular Matrix, analysis. Fibronectins, analysis. Fibronectins, biosynthesis. Glycoproteins, analysis. Human. Immunoenzyme Techniques. Infant, Newborn. Laminin, analysis. Laminin, biosynthesis. Support, Non-U.S. Gov't. Support, U.S. Gov't, P.H.S.. Thrombospondins.

**RNUM:** 0 (Fibronectins); 0 (Glycoproteins); 0 (Laminin); 0 (Thrombospondins); 9007-34-5 (Collagen)

**GEOT:** UNITED STATES

**IDEN:** ISSN: 0021-9541. JOURNAL-CODE: 0050222. ENTRY-DATE: 19850415. NIH-GRANT-NUMBER: AG 01312/AG/NIA. CA 33834/CA/NCI. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

**PMID:** 3882725

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Terms: **author (kramer and bensch)** ([Edit Search](#))

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Terms: author (sontheimer) and title(dendritic) ([Edit Search](#))

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**TITL:** Perivascular **dendritic** macrophages as immunobiological constituents of the human dermal microvascular unit. (43 REFS)

**AUTH:** Sontheimer R D

**ORGA:** Department of Dermatology and Internal Medicine, University of Texas Southwestern Medical Center, Dallas 75235.

**PUB TYPE:** Journal Article. Review. Review, Tutorial.

**CITE:** J Invest Dermatol 1989 Aug; 93 (2 Suppl): 96S-101S

**LANG:** ENG; English

**ABST:** We have recently observed a previously uncharacterized population of class II (Ia) antigen-positive dendritic cells that is intimately associated with the dermal microvessels of normal human adult and newborn skin. Immunofluorescence and immunoelectron microscopy studies have indicated that this cell is a perivascular macrophage. This dermal perivascular dendritic macrophage was seen in greatest density in the superficial vascular plexus and appears to constitute a large percentage of the total dermal macrophage population. Because tissue macrophages do not always share the same immunologic repertoire as circulating monocytes/macrophages, we became interested in examining the functional immunologic capabilities of this dermal dendritic macrophage. A procedure was developed to isolate and partially purify these cells from normal human newborn foreskin. Preliminary findings are compatible with the possibility that these cells possess the capacity to present alloantigens to CD4+ T cells. The potential immunologic function of this dermal perivascular macrophage is discussed in the context of its possible interactions with the other cellular components of the dermal microvascular unit (i.e., microvascular endothelial cells, perivascular T cells, and mast cells).


**MJTR:** Dendritic Cells, immunology. Endothelium, Vascular, immunology. Macrophages, immunology. Skin, blood supply.


**MNTR:** Animals. Dendritic Cells, physiology. Endothelium, Vascular, cytology. Human. Macrophages, physiology. Microcirculation. Phenotype. Support, Non-U.S. Gov't. Support, U.S. Gov't, P.H.S.. T-Lymphocytes, immunology. T-Lymphocytes, physiology.

**GEOT:** UNITED STATES

**IDEN:** ISSN: 0022-202X. JOURNAL-CODE: 0426720. ENTRY-DATE: 19890901. NIH-GRANT-NUMBER: AM09989/AM/NIADDK. AR01784/AR/NIAMS. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

**PMID:** 2666528

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Terms: author (zhang and kramer) and title (laminin) ([Edit Search](#))

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**TITL:** Laminin 5 deposition promotes keratinocyte motility.

**AUTH:** Zhang K; Kramer R H

**ORGA:** Department of Stomatology, University of California at San Francisco 94143-0512, USA.

**PUB TYPE:** Journal Article.

**CITE:** Exp Cell Res 1996 Sep 15; 227 (2): 309-22

**LANG:** ENG; English

**ABST:** We examined the role of individual integrins in promoting human keratinocyte migration. In short-term assays on collagen type I- or fibronectin-coated substrates, migration was blocked by antibody to the alpha 2 integrin and the alpha 5 integrin, respectively. Unexpectedly, antibodies to integrin alpha 3 also significantly inhibited cell locomotion on both ligands. Time-course immunofluorescence staining revealed that keratinocyte migration was accompanied by deposition of endogenous laminin 5. Since alpha 3 beta 1 is a known receptor for this ligand, this observation suggested that migrating keratinocytes use freshly deposited laminin 5 in locomotion. Indeed, further investigation showed that anti-laminin 5 blocking antibodies effectively inhibited keratinocyte motility on both collagen and fibronectin substrates. Furthermore, cell migration on laminin 5-coated substrates was blocked by both anti-alpha 3 and anti-laminin 5 antibodies. Laminin 5 did not appear important in the initial attachment of keratinocytes, since adhesion of cells to collagen type I- or fibronectin-coated surfaces was not blocked by antibody to alpha 3 integrin or to laminin 5, but could be inhibited by antibody to alpha 2 or alpha 5, respectively. Using an in vitro wound assay, blocking antibodies to alpha 3 integrin and to laminin 5 also blocked reepithelization of the denuded monolayer. These results show that alpha 3 beta 1 integrin plays an important role in the migration of keratinocytes via their interaction with laminin 5. Furthermore, they suggest that cell migration is dependent not only on exogenous ligands but, importantly, on endogenously secreted laminin 5. Finally, the data are consistent with our earlier finding that laminin 5 is the first extracellular matrix component to be expressed and deposited by migrating keratinocytes during wound healing in vivo.

**MJTR:** Cell Adhesion Molecules, metabolism. Cell Movement, physiology. Keratinocytes, chemistry. Keratinocytes, cytology.

**MNTR:** Cell Adhesion, physiology. Cell Adhesion Molecules, analysis. Human. Infant, Newborn. Integrins, physiology. Keratinocytes, physiology. Male. Skin, cytology. Support, U.S. Gov't, P.H.S..

**RNUM:** 0 (Cell Adhesion Molecules); 0 (Integrins); 0 (kalinin)

**GEOT:** UNITED STATES

**IDEN:** ISSN: 0014-4827. JOURNAL-CODE: 0373226. ENTRY-DATE: 19961105. NIH-GRANT-NUMBER: DE10306/DE/NIDCR. DE11436/DE/NIDCR. DE11912/DE/NIDCR. etc. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

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Terms: **author (hansbrough and morgan)** ([Edit Search](#))

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**TITLE:** Composite grafts of human keratinocytes grown on a polyglactin mesh-cultured fibroblast dermal substitute function as a bilayer skin replacement in full-thickness wounds on athymic mice.

**AUTH:** Hansbrough J F; Morgan J L; Greenleaf G E; Bartel R

**ORGA:** Department of Surgery, University of California, San Diego Medical Center 92103.

**PUB TYPE:** Journal Article.

**CITE:** J Burn Care Rehabil 1993 Sep-Oct; 14 (5): 485-94

**LANG:** ENG; English

**ABST:** We have developed and tested in athymic mice a new, cultured, dermal-epidermal graft composed of two human cell types coupled with a biodegradable dermal scaffold. Cultured, proliferating human keratinocytes (HK) were applied to the surface of a living dermal tissue replacement that is composed of human fibroblasts cultured on a polyglactin mesh. After 4 to 6 days of coculture, proliferating HKs achieved confluency on the surface of the living dermal tissue replacement. Grafts were then transferred to full-thickness wounds on the dorsum of athymic mice. Sixteen animals were grafted, and the mean percentage of graft take (original wound area covered) on day 20 after grafting was 51.25%. Staining with antibody specific for human involucrin confirmed the presence of HKs on closed wounds, and staining with antibody specific for human laminin revealed a continuous layer of laminin at the dermal-epidermal junction on day 20. Animals closed with living dermal tissue replacement alone markedly contracted, whereas application of living dermal tissue replacement-HK grafts appeared to retard contraction. Because polyglactin mesh fibers are absorbed by hydrolysis rather than by enzymatic degradation, this living composite graft may be more resistant to destruction when placed on excised human wounds than are composite grafts, which are composed of a collagen matrix. The inclusion of the living dermal substitute may ultimately provide better skin quality than is achieved from the use of cultured keratinocytes alone. Fragility of the epidermal layer is probably due to the short-term culture of HKs on the living dermal tissue replacement, and further efforts to develop a thicker epithelial layer may improve graft durability.

**MJTR:** Keratinocytes. Polyglactin 910. Skin Transplantation, methods. Skin, Artificial. Wound Healing, physiology.

**MNTR:** Animals. Cells, Cultured. Fibroblasts. Graft Survival, physiology. Human. Mice. Mice, Nude. Surgical Mesh.

**RNUM:** 34346-01-5 (Polyglactin 910)

**GEOT:** UNITED STATES

**IDEN:** ISSN: 0273-8481. JOURNAL-CODE: 8110188. ENTRY-DATE: 19931227. SPECIAL-LIST: IM N. JOURNAL-SUBSET: IM N.

**PMID:** 8245102



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Terms: **author (kangesu and manek)** ([Edit Search](#))

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**TITL:** Kerato-dermal grafts: the importance of dermis for the in vivo growth of cultured keratinocytes.

**AUTH:** Kangesu T; Navsaria H A; Manek S; Fryer P R; Leigh I M; Green C J

**ORGA:** RAFT, Department of Research in Plastic Surgery, Mount Vernon Hospital NHS Trust, Northwood, Middx., UK.

**PUB TYPE:** Journal Article.

**CITE:** Br J Plast Surg 1993 Jul; 46 (5): 401-9

**LANG:** ENG; English

**ABST:** In a porcine model, we studied the benefit of dermis for the growth of cultured autologous keratinocytes (CAK) on full-thickness wounds isolated within skin graft chambers. Kerato-dermal grafts were prepared in a two stage process using autologous de-epidermalised dermis (DED) and CAK (Group 1). Control wounds were prepared by grafting either CAK only (Group 2) or DED only (Group 3). The median epidermal cover of 34 wounds in Group 1 was 47% and was significantly greater ( $p < 0.001$ ) than the epidermal cover of 12 wounds in Group 2 (4%) and 14 wounds in Group 3 (12%). The epidermis in Group 1 was durable whereas it was fragile in the control wounds. Histologically rete ridges were present at 2 weeks in Group 1, but not in the control wounds. These data indicate that a dermal wound bed significantly improves the in vivo growth of cultured keratinocytes.

**MJTR:** Keratinocytes, transplantation. Keratinocytes, ultrastructure. Skin Physiology. Skin Transplantation, physiology.

**MNTR:** Animals. Cells, Cultured. Collagen, analysis. Epidermis, ultrastructure. Immunohistochemistry. Microscopy, Electron. Pilot Projects. Skin, chemistry. Support, Non-U.S. Gov't. Swine.

**RNUM:** 9007-34-5 (Collagen)

**GEOT:** SCOTLAND

**IDEN:** ISSN: 0007-1226. JOURNAL-CODE: 2984714R. ENTRY-DATE: 19931014. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

**PMID:** 8369878

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Terms: **author (kangesu and manek)** ([Edit Search](#))

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Terms: **author (keating and salo)** ([Edit Search](#))

*National Library of Medicine MEDLINE Database*

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**TITLE:** Donor origin of the in vitro haematopoietic microenvironment after marrow transplantation in man.

**AUTH:** **Keating** A; Singer J W; Killen P D; Striker G E; **Saló** A C; Sanders J; Thomas E D; Thorning D; Fialkow P J

PUB TYPE: Journal Article.

**CITE:** Nature 1982 Jul 15; 298 (5871): 280-3

**LANG:** ENG; English

**ABST:** The method for long-term culture of marrow cells in vitro as described by Dexter has recently been successfully applied to human marrow and is dependent on the development of an adherent stromal cell layer consisting of cells described as "endothelial-like cells, fat cells, and macrophages". The present study was designed to determine the origin and composition of the stromal cells forming the in vitro 'microenvironment' and maintaining haematopoiesis in long-term cultures grown from marrows of 14 patients who received marrow transplants from HLA identical siblings of the opposite sex. The presence of a Y chromosome was used as a marker to establish the donor or recipient origin of the cells. We found that the stromal cells became progressively donor in origin with time after transplantation and some reacted with antibody directed against factor VIII-associated antigen. In addition, donor-derived in vitro stromal cells synthesized both interstitial and basal lamina collagen types, indicating that the in vitro microenvironment is transplantable and composed in part of endothelial-like cells.

**MJTR:** Bone Marrow Transplantation. Hematopoiesis.


**MNTR:** Adipose Tissue, cytology. Antigens, immunology. Bone Marrow, metabolism. Cells, Cultured. Collagen, biosynthesis. Epithelial Cells. Epithelium, immunology. Factor VIII, immunology. Female. Graft Survival. Human. Karyotyping. Macrophages. Male. Support, Non-U.S. Gov't. Support, U.S. Gov't, P.H.S.. Y Chromosome. von Willebrand Factor.

**RNUM:** 0 (Antigens); 0 (von Willebrand Factor); 9001-27-8 (Factor VIII); 9007-34-5 (Collagen)

**GEOT:** ENGLAND

**IDEN:** ISSN: 0028-0836. JOURNAL-CODE: 0410462. ENTRY-DATE: 19820826. NIH-GRANT-NUMBER: A1 01425/PHS. CA 16448/CA/NCI. CA 18029/CA/NCI. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

**PMID:** 6806668

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Terms: **author (keating and salo)** ([Edit Search](#))

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Terms: author (benezra and neufeld) ([Edit Search](#))

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**TITL:** Thrombin-induced release of active basic fibroblast growth factor-heparan sulfate complexes from subendothelial extracellular matrix.

**AUTH:** Benezra M; Vlodavsky I; Ishai-Michaeli R; Neufeld G; Bar-Shavit R

**ORGA:** Department of Oncology, Hadassah-Hebrew University Hospital, Jerusalem, Israel.

**PUB TYPE:** Journal Article.

**CITE:** Blood 1993 Jun 15; 81 (12): 3324-31

**LANG:** ENG; English

**ABST:** The angiogenic factor, basic fibroblast growth factor (bFGF), is sequestered and protected by binding to heparan sulfate proteoglycans (HSPG) in the subendothelial extracellular matrix (ECM). Release of ECM-bound bFGF provides a novel mechanism for regulation of cell proliferation and neovascularization in normal and pathologic situations. Exposure of ECM to thrombin, the final activation product of the clotting cascade, resulted in release of high molecular weight HSPG-bFGF complex, as indicated by its immunoprecipitation with anti-bFGF antibodies, susceptibility to degradation by bacterial heparinase, and inhibition of its mitogenic activity in the presence of neutralizing anti-bFGF antibodies. The ECM-resident bFGF-HSPG complex was not released by thrombin in the presence of hirudin or antithrombin III, or by catalytically blocked thrombin preparations. A threefold to fivefold higher mitogenic activity was released by thrombin from ECM that was preheated (1 hour, 80 degrees C), as compared with native ECM. This difference is attributed to heat stable bFGF-HSPG complexes that are more readily released after heat treatment of the ECM and to activation and release of ECM-resident transforming growth factor-beta (TGF-beta) activity. Our results indicate that the large reservoir of proteolytic activity present in plasma in the form of prothrombin may participate in release from the subendothelial ECM of biologically active bFGF and TGF-beta, depending on the accessibility of thrombin. Thrombin may gain access to the subendothelium on clot formation after tissue injury and as a result of the conversion of prothrombin to thrombin induced by the ECM itself.


**MJTR:** Extracellular Matrix, secretion. Fibroblast Growth Factor 2, secretion. Heparitin Sulfate, secretion. Thrombin, pharmacology.

**MNTR:** Animals. Antithrombin III, pharmacology. Cattle. Cell Division. Cornea. Endothelium, metabolism. Extracellular Matrix, drug effects. Fibroblast Growth Factor 2, metabolism. Heat. Heparin Lyase. Heparitin Sulfate, metabolism. Hirudin, pharmacology. Human. Immunosorbent Techniques. Male. Molecular Weight. Polysaccharide-Lyases, metabolism. Support, Non-U.S. Gov't. Support, U.S. Gov't, Non-P.H.S.. Thrombin, antagonists & inhibitors.

**RNUM:** 103107-01-3 (Fibroblast Growth Factor 2); 8001-27-2 (Hirudin); 9000-94-6 (Antithrombin III); 9050-30-0 (Heparitin Sulfate); EC 3.4.21.5 (Thrombin); EC 4.2.2. (Polysaccharide-Lyases); EC 4.2.2.7 (Heparin Lyase)

**GEOT:** UNITED STATES

**IDEN:** ISSN: 0006-4971. JOURNAL-CODE: 7603509. ENTRY-DATE: 19930714. SPECIAL-LIST: AIM IM. JOURNAL-SUBSET: AIM IM.

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Terms: **author (normand and karasek)** ([Edit Search](#))

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**TITLE:** A method for the isolation and serial propagation of keratinocytes, endothelial cells, and fibroblasts from a single punch biopsy of human skin.

**AUTH:** Normand J; Karasek M A

**ORGA:** Department of Dermatology, Stanford University School of Medicine, California 94305, USA.

PUB TYPE: Journal Article.

**CITE:** In Vitro Cell Dev Biol Anim 1995 Jun; 31 (6): 447-55

**LANG:** ENG; English

**ABST:** When multiple types of cells from normal and diseased human skin are required, techniques to isolate cells from small skin biopsies would facilitate experimental studies. The purpose of this investigation was to develop a method for the isolation and propagation of three major cell types (keratinocytes, microvascular endothelial cells, and fibroblasts) from a 4-mm punch biopsy of human skin. To isolate and propagate keratinocytes from a punch biopsy, the epidermis was separated from the dermis by treatment with dispase. Keratinocytes were dissociated from the epidermis by trypsin and plated on a collagen-coated tissue culture petri dish. A combination of two commercial media (Serum-Free Medium and Medium 154) provided optimal growth conditions. To isolate and propagate microvascular endothelial cells from the dermis, cells were released following dispase incubation and plated on a gelatin-coated tissue culture dish. Supplementation of a standard growth medium with a medium conditioned by mouse 3T3 cells was required for the establishment and growth of these cells. Epithelioid endothelial cells were separated from spindle-shaped endothelial cells and from dendritic cells by selective attachment to Ulex europeus agglutinin I-coated paramagnetic beads. To establish fibroblasts, dermal explants depleted of keratinocytes and endothelial cells were attached to plastic by centrifugation, and fibroblasts were obtained by explant culture and grown in Dulbecco's modified Eagle's medium (DMEM) containing fetal bovine serum (FBS). Using these isolation methods and growth conditions, two confluent T-75 flasks of keratinocytes, one confluent T-25 flask of purified endothelial cells, and one confluent T-25 flask of fibroblasts could be routinely obtained from a 4-mm punch biopsy of human skin.(ABSTRACT TRUNCATED AT 250 WORDS)

**MJTR:** Cell Culture, methods. Endothelium, pathology. Fibroblasts, pathology. Keratinocytes, pathology. Skin, pathology.

**MNTR:** 3T3 Cells. Animals. Biopsy. Culture Media. Human. Mice. Mice, Inbred BALB C. Support, U.S. Gov't, P.H.S..

**RNUM:** 0 (Culture Media)

**GEOT:** UNITED STATES

**IDEN:** ISSN: 1071-2690. JOURNAL-CODE: 9418515. ENTRY-DATE: 19960325. NIH-GRANT-NUMBER: AR 41045/AR/NIAMS. HL 48495/HL/NHLBI. SPECIAL-LIST: IM. JOURNAL-SUBSET: IM.

**PMID:** 8589888